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*January 11, 2005*

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**APPLICATION NUMBER: 60/466,398**

**FILING DATE: April 29, 2003**

**RELATED PCT APPLICATION NUMBER: PCT/US04/12882**



**Certified By**

**Jon W Dudas**

**Under Secretary  
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04/29/03

JCS53 U.S. PTO

04-30-03 60/466398 .042903

Practitioner's Docket No. CCF-6566PV

PATENT

Approved  
\$

## Preliminary Classification:

Proposed Class:

Subclass:

Note: "All applicants are requested to include a preliminary classification on newly filed patent applications. The preliminary classification, preferably class and subclass designations, should be identified in the upper right-hand corner of the letter of transmittal accompanying the application papers, for example 'Proposed Class 2, subclass 129.'" M.P.E.P. § 601, 7<sup>th</sup> ed.

## IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Gary W. Procop

For: REAL-TIME PCR FOR THE DETECTION OF ALL SALMONELLA SPECIES (PAN-SALMONELLA) PCR, WITH DIFFERENTIATION OF S. TYPHI FROM NON-TYPHI SALMONELLA

Box Provisional Patent Application  
Assistant Commissioner for Patents  
Washington, D.C. 20231

JC996 U.S. PTO  
60/466398  
04/29/03COVER SHEET FOR FILING PROVISIONAL APPLICATION  
(37 C.F.R. § 1.51(c)(1))

**WARNING:** "A provisional application must also include the cover sheet required by § 1.51(c)(1) or a cover letter identifying the application as a provisional application. Otherwise, the application will be treated as an application filed under paragraph (b) [nonprovisional application] of this section." 37 C.F.R. § 1.53(c)(1). See also M.P.E.P. § 201.04(b), 6th ed., rev. 3.

## CERTIFICATE OF MAILING/TRANSMISSION 37 CFR § 1.10\*

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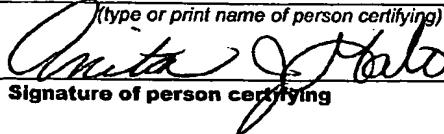
## CERTIFICATE OF MAILING/TRANSMISSION 37 CFR §1.10\*

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Date: April 29, 2003

Anita J. Galo

(type or print name of person certifying)



Signature of person certifying

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"Since the filing of correspondence under § 1.10 without the Express Mail mailing label thereon is an oversight that can be avoided by the exercise of reasonable care, requests for waiver of this requirement will not be granted on petition." Notice of Oct. 24, 1996, 60 Fed. Reg. 56, 439, at 56, 442.

NOTE: "A complete provisional application does not require claims since no examination on the merits will be given to a provisional application. However, provisional applications may be filed with one or more claims as part of the application. Nevertheless, no additional claim fee or multiple dependent claims fee will be required in a provisional application." Notice of December 5, 1994, 59 Fed. Reg. 63,951, at 63,953. "Any claim filed with a provisional application will, of course, be considered part of the original provisional application disclosure." Notice of April 14, 1995, 60 Fed. Reg. 20,195, at 20,209.

NOTE: "A provisional application is not entitled to the right of priority under 35 U.S.C. 119 or 365(a) or § 1.55, or to the benefit of an earlier filing date under 35 U.S.C. 120, 121 or 365(c) or § 1.78 of any other application. No claim for priority under § 1.78(a)(3) may be made in a design application based on a provisional application. No request under § 1.293 for a statutory invention registration may be filed in a provisional application. The requirements of §§ 1.821 through 1.825 regarding application disclosures containing nucleotide and/or amino acid sequences are not mandatory for provisional applications." 37 C.F.R. § 1.53(c)(3).

NOTE: "No information disclosure statement may be filed in a provisional application." 37 C.F.R. § 1.51(d). "Any information disclosure statements filed in a provisional application would either be returned or disposed of at the convenience of the Office." Notice of December 5, 1994, 59 Fed. Reg. 63,591, at 63,594.

NOTE: "No amendment other than to make the provisional application comply with the patent statute and all applicable regulations may be made to the provisional application after the filing date of the provisional application." 37 C.F.R. § 1.53(c).

NOTE: 35 U.S.C. 119(e) provides that "[i]f the day that is 12 months after the filing date of a provisional application falls on a Saturday, Sunday, or Federal Holiday within the District of Columbia, the period of pendency of the provisional application shall be extended to the next succeeding secular or business day".

**This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 C.F.R. § 1.51(c)(1)(i).**

1. The following comprises the information required by 37 C.F.R. § 1.51(c)(1):

2. The name(s) of the inventor(s) is/are (37 C.F.R. § 1.51(c)(1)(ii):

NOTE: "If the correct inventor or inventors are not named on filing a provisional application without a cover sheet under § 1.51(c)(1), the later submission of a cover sheet under § 1.51(c)(1) during the pendency of the application will act to correct the earlier identification of inventorship." 37 C.F.R. § 1.48(f)(2).

NOTE: "The naming of inventors for obtaining a filing date for a provisional application is the same as for other applications. A provisional application filed with the inventors identified as 'Jones et al.' will not be accorded a filing date earlier than the date upon which the name of each inventor is supplied unless a petition with the fee set forth in § 1.17(i) is filed which sets forth the reasons the delay in supplying the names should be excused. Administrative oversight is an acceptable reason. It should be noted that for a 35 U.S.C. 111(a) application to be entitled to claim the benefit of the filing date of a provisional application the 35 U.S.C. 111(a)[.] application must have at least one inventor in common with the provisional application." Notice of April 14, 1995, 60 Fed. Reg. 20,195, at 20,209.

The term "invention" is typically used to refer to subject matter which applicant is claiming in his/her application. Because claims are not required in a provisional application, it would not be appropriate to reference joint inventors as those who have made a contribution to the "invention" disclosed in the provisional application. If the "invention" has not been determined in the provisional application because no claims have been presented, then the name(s) of those person(s) who have made a contribution to the subject matter disclosed in the provisional application should be submitted. Section 1.45(c) states that "if multiple inventors are named in a provisional application, each named inventor must have made a contribution, individually or jointly, to the subject matter disclosed in the provisional application." All that § 1.45(c) requires is that if someone is named as an inventor, that person must have made a contribution to the subject matter disclosed in the provisional application. When applicant has determined what the invention is by the filing of the 35 U.S.C. 111(a) application, that is the time when the correct inventors must be named. The 35 U.S.C. 111(a) application must have an inventor in common with the provisional application in order for the 35 U.S.C. 111(a) application to be entitled to claim the benefit of the provisional application under 35 U.S.C. 119(e). Notice of April 14, 1995, 60 Fed. Reg. 20,195, at 20,208.

See 37 C.F.R. § 1.53.



## 9. Identification of documents accompanying this cover sheet.

## A. Documents required by 37 C.F.R. §§ 1.51(c)(2)-(3):

Specification: No. of pages EIGHT (8)  
 Drawings: No. of sheets THREE (2) (Figs. 1-3)

## B. Additional documents:

☒ Claims: No. of claims FIVE (5) (ONE (1) Pages)  
☒ Abstract No. of pages N/A

Note: See 37 C.F.R. § 1.51.

- ☐ Power of attorney  
☐ Small entity assertion  
☐ Assignment  
☐ English language translation of non-English provisional application

NOTE: A provisional application which is filed in a language other than English, does not have to have an English language translation. See 37 C.F.R. § 1.52(d)(2). However, if the provisional application is not in the English language and will later serve as a benefit of its filing date for a nonprovisional application, other than a design patent, or for an international application designating the U.S., then an English language translation must be filed in the provisional application or the later filed nonprovisional application. See § 1.78(e)(5)(iv).

- ☐ This application is in a language other than English and an English translation along with a statement of its accuracy is submitted herewith.  
☐ Other

## 10. Fee

The filing fee for this provisional application, as set in 37 C.F.R. § 1.16(k), is \$160.00, for other than small entity, and \$80.00, for a small entity.

- ☒ Applicant is a small entity.

NOTE: "A . . . statement in compliance with existing § 1.27 is required to be filed in each provisional application in which it is desired to pay reduced fees." Notice of April 14, 1995, 60 Fed. Reg. 20,195, at 20,197.

## 11. Small entity statement assertion

- ☐ The assertion that this is a filing by a small entity under 37 C.F.R. § 1.27 (c)(1) is attached ("ASSERTION OF SMALL ENTITY STATUS").  
☒ Small entity status is asserted for this application by payment of the small entity filing fee under § 1.16(k). 37 C.F.R. § 1.27(c)(3).

## 12. Fee payment

- ☒ Fee payment in the amount of \$80.00 is being made at this time.  
☐ No filing fee is to be paid at this time (This and the surcharge required by 37 C.F.R. § 1.16(l) can be paid subsequently).

**13. Method of fee payment**

- ☒ Attached is a ☒ check ☐ money order in the amount of \$80.00
- ☒ Authorization is hereby made to charge the amount of \$0.00
- ☒ to Deposit Account No. 20-090.
- ☐ to Credit card as shown on the attached credit card information authorization form PTO-2038.

**WARNING:** Credit card information should not be included on this form as it may become public.

- ☒ Charge any additional fees required by this paper or credit any overpayment in the manner authorized above.

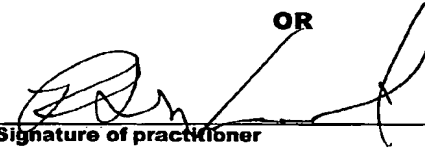
A duplicate of this paper is attached.

Date:

\_\_\_\_\_  
Signature of submitter

Tel.:

Date: April 29, 2003

**OR**  
  
\_\_\_\_\_  
Signature of practitioner

Reg. No. 40,871

Richard S. Wesorick  
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## PROVISIONAL PATENT

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SIGNATURE OF PERSON MAILING PAPER OR FEE

Anita S. Galo

NAME OF PERSON SIGNING

April 29, 2003

DATE OF SIGNATURE

**REAL-TIME PCR FOR THE DETECTION OF ALL  
SALMONELLA SPECIES (PAN-SALMONELLA) PCR, WITH  
DIFFERENTIATION OF *S. TYPHI* FROM NON-TYPHI *SALMONELLA***

**BACKGROUND OF THE INVENTION**

*Salmonella* can be identified from stool using cultures. Culture for *Salmonella* species from stool is time-consuming and lacks sensitivity. For the identification of *Salmonella* isolates from a stool culture, primary screening agar plates are used, sometimes in conjunction with enrichment broths. Suspect isolates, which are often present are screened using additional screening agars (The triple sugar iron agar, lysine iron agar, and urea agar). Biochemical identification is then performed, and, if positive, serologic confirmation is used. Because only approximately 2% of cultured stools contain *Salmonella*, an incredible amount of work occurs due to bacterial "look-alikes" (lactose negative members of the *Enterobacteriaceae*).

*Salmonella* species are identified from positive blood cultures by routine biochemical methods. In developing countries, including countries where U.S. troops may be involved, typhoid and paratyphoid fever (enteric fever) is endemic. The rapid identification of *Salmonella* directly from the blood specimen may save two days over current technology, and help distinguish enteric fever from other tropical febrile illnesses, such as malaria.

One method regarding the detection of *Salmonella* on poultry involves real-time PCR. The *invA* gene has been used for the detection of *Salmonella* strains in feces. Other PCR assays

for *Salmonella* target the 16S ribosomal subunit gene complex or genes that encode flagellar antigens. These assays however do not differentiate the *S. typhi* isolates from the non-Typhi *Salmonella*. A variety of PCR assays have been used for detection of *S. typhi*, including some that target genes that encode the Vi antigen. Real-time formats for the detection of *S. typhi* by the detection of the *vexC* gene however have not been described.

### **SUMMARY OF THE INVENTION**

One aspect of the invention relates to a portion of nucleic acid that is useful for detection of all *Salmonella* strains and differentiation of these strains from other bacteria. In addition, this portion of nucleic acid contains genetic information useful for the differentiation of *S. typhi* from non-Typhi *Salmonella*.

Another aspect of the invention relates to a real-time PCR assay that detects a specific portion of the organism's genome, a portion of the *prgK* gene, which we have shown is useful for the detection of all *Salmonella*, differentiation of *Salmonella* from other bacteria, and differentiation of *Salmonella* into Typhi and non-Typhi groups by melt curve analysis.

A further aspect of the invention relates to a portion of nucleic acid that affords the specific detection of only *S. typhi* from all other bacteria, including other *Salmonella* isolates.

Yet a further aspect of the invention relates to a real-time PCR assay that detects only *S. typhi*, by the detection of the *vexC* gene, a portion of the gene complex associated with the Vi antigen.

### **BRIEF DESCRIPTION OF THE DRAWINGS**

The foregoing and other features of the present invention will become apparent to one skilled in the art to which the present invention relates upon reading the following description with reference to the accompanying drawings.

FIG. 1 is a graph illustrating all *Salmonella* isolates that are detected by the Pan-*Salmonella* assay.

FIG. 2 is a graph illustrating that melting point analysis differentiates the *Salmonella* into three groups: *S. typhi* (left), *S. typhimurium* (right), and all other *Salmonella* (center).

FIG. 3 is a graph illustrating that the *S. typhi* specific PCR only detects *S. typhi*.



### DETAILED DESCRIPTION

The present invention relates generally to a portion of nucleic acid that is useful for detection of all *Salmonella* strains and differentiation of these strains from other bacteria. In addition, this portion of nucleic acid contains genetic information useful for the differentiation of *S. typhi* from non-Typhi *Salmonella*. The present invention also relates generally to a portion of nucleic acid that affords the specific detection of only *S. typhi* from all other bacteria, including other *Salmonella* isolates.

These portions of DNA are amplified by PCR using PCR primers, and the detection and differentiation of all *Salmonella* isolates (for the pan-*Salmonella* PCR) and *S. typhi* (for the *S. typhi* PCR) is achieved using fluorescently-labelled hybridization probes. This assay is performed using real-time PCR in the LightCycler system, employing fluorescent resonance energy transfer (FRET) and differential melting temperature technology. This system is commercially-available, but only limited assays for this system are commercially marketed by the company (Roche). The same primers/probes could be used in other real-time PCR systems.

In brief, the pan-*Salmonella* PCR amplifies and detects all clinically-relevant isolates of *Salmonella* (FIG. 1). The hybridization probes are used primarily for the detection of the amplified product, but also afford differentiation of *S. typhi* from non-Typhi *Salmonella* by melting point analysis (FIG. 2). Melting point analysis determines the temperature at which the hybridization probes disassociate or melts off the target DNA sequence. Melting point analysis, therefore, is dependent upon the nucleotides present in the DNA sequence. We have found that the melting point differs between the *S. typhi* and other *Salmonella* species in the pan-*Salmonella* real-time PCR assay. Although we proved this differentiation with cultured isolates, we considered that when employed, users may desire a confirmatory assay for *S. typhi* (FIG. 3). Therefore, we constructed a real-time PCR that amplified and detects a portion of the gene that encodes the Vi antigen. This assay also is dependent upon the sequence of the target nucleic acid. We have identified a nucleic acid sequence that makes such an assay possible, and constructed primers and hybridization probes that makes this assay possible.

### The Pan-Salmonella Assay

A variety of genes of *Salmonella* are associated with cellular invasion. We targeted a portion of one of these genes for the pan-*Salmonella* PCR, the *prgK* gene. The GenBank entry used was AE008831 (*S. typhimurium*). The *prgK* gene is thought to encode a lipoprotein that links inner and outer membrane proteins of this complex. The *prgK* is located from basepair 4139 to basepair 4897 (GenBank # AE008831, underlined portion of SEQ NO. 1 listed below). The portion of the *prgK* gene used for this assay is located from basepair 4179 to basepair 4372. In *Styphi* (GenBank # AL627276), the location of the *prgK* gene is 165010 to 165768 and is distinct from the *invA* gene, which has been previously used for *Salmonella* PCR, and is located from 188400 to 190457. Other regions within this gene complex would likely also be suitable for the development of a similar assay.

### The *S. typhi* PCR assay

There are a variety of genes that encode or are associated with the Vi antigen of *S. typhi*. We chose one of these genes, the *vexC* gene, as the target for the real-time *S. typhi* PCR. This product of this gene is thought to be a polysaccharide ATP binding protein. The *vexC* is located from basepair 41900 to basepair 42595 (GenBank # AL627283, underlined portion of SEQ NO. 2 listed below). The portion of the *vexC* gene used for this assay is located from basepair 42395 to basepair 42595. Other regions within this gene complex would likely also be suitable for the development of a similar assay.

### Pan-Salmonella Assay

Forward Primer:	5'-CCTTTCTTATTGCGGGCA-3'
Reverse Primer:	5'-GCCGATGTGGATTATGAC-3'
Hybridization Probe 1:	5'-GGATTGTTTTGATTATTTTGTTATCCGTGATG-FITC-3'
Hybridization Probe 2:	5'- LCRed705-AGCAGGCTTTGGCGT-P-3'

### *Salmonella typhi* PCR

Forward Primer:	5'-ACCCCGTAGCCCAATA-3'
Reverse Primer:	5'-AGGAGAGACGCATTCG-3'
Hybridization Probe 1:	5'-GCATATCGGTATTCTGGCGGC-FITC-3'
Hybridization Probe 2:	5'- LCRed640-CTGGTTCAGGCAAAACGACG-P-3'

**TABLE 1**

	GenBank Number	Position	Target
Pan-Salmonella Forward Primer	AE008831	4179-4196	PrgK gene
Pan-Salmonella Reverse Primer	AE008831	4372-4355	PrgK gene
Pan-Salmonella Hybridization Probe 1	AE008831	4266-4201	PrgK gene
Pan-Salmonella Hybridization Probe 2	AE008831	4179-4196	PrgK gene
<i>S. typhi</i> Forward Primer	AL627283	42395-42410	VexC gene
<i>S. typhi</i> Reverse Primer	AL627283	42610-42595	VexC gene
<i>S. typhi</i> Hybridization Probe 1	AL627283	42500-42521	VexC gene
<i>S. typhi</i> Hybridization Probe 2	AL627283	42524-42458	VexC gene

We have tested 274 bacterial isolates, of which 101 were various strains of *Salmonella* (TABLE 2). The *Salmonella* isolates tested included 23 strains of *S. typhi*, 24 strains of *S. paratyphi*, 15 strains of *S. typhimurium*, 6 strains of *S. enteritidis*, and 5 strains of *S. choleraesuis*. Other important enteric pathogens tested included 35 strains of *Shigella*, representing all four species, 27 strains of *Yersinia enterocolitica*, 12 strains of *E. coli* O157:H7, and a single *Campylobacter jejuni* isolate. The remainder of the organisms tested consisted of a variety of bacteria that may be present in clinical specimens and isolated in the clinical microbiology laboratory.

The pan-*Salmonella* assay correctly detected all isolates of *Salmonella* tested and the melting curve of *S. typhi* was distinctive from the melt curves of other *Salmonella* species. The *S. typhi* assay was positive only for the isolates of *S. typhi*. There was no cross-reactivity with the other bacteria tested.

**TABLE 2**

Light Cycler Results  
Bacterial PCR Test Battery vs Pan Salmonella and Styphi  
Hybridization Probes

Organism (n)	Pan Salm LC result	Styphi LC result	Organism (n)	Pan Salm LC result	Styphi LC result
Staph aureus (5)	-	-	Providencia (2)	-	-
Staph epidermidis (3)	-	-	Shigella sonnei (10)	-	-
Staph saprophyticus (2)	-	-	Shigella flexneri, Group B (17)	-	-
Micrococcus (2)	-	-	Shigella boydii, Group C (6)	-	-
Stomatococcus (2)	-	-	Shigella dysenteriae, Group A (2)	-	-
Lactobacillus (2)	-	-	Burkholderia (2)	-	-
Enterococcus (3)	-	-	Yersinia kristensenii	-	-
Viridans streptococcus (3)	-	-	Yersinia enterocolitica (27)	-	-
Strep pneumoniae (3)	-	-	Citrobacter (3)	-	-
Group A streptococcus (3)	-	-	E. coli (2)	-	-
Group B streptococcus (3)	-	-	E. coli 0157 (12)	-	-
Aerococcus (3)	-	-	Proteus (3)	-	-
Listeria (3)	-	-	Klebsiella (3)	-	-
Bacillus (3)	-	-	Enterobacter (3)	-	-
Salmonella typhimurium (15)	+	-	Pseudomonas (3)	-	-
Salmonella enteritidis (6)	+	-	Acinetobacter (3)	-	-
Salmonella typhi (23)	+	+	Haemophilus (3)	-	-
Salmonella choleraesuis (5)	+	-	Neisseria meningitidis (3)	-	-
Salmonella paratyphi (24)	+	-	Neisseria gonorrhoea (3)	-	-
Salmonella agona	+	-	Non-gonococcal Neisseria sp (3)	-	-
Salmonella oslo	+	-	Moraxella (3)	-	-
Salmonella poona	+	-	Bacteroides (3)	-	-
Salmonella heidelberg (5)	+	-	Afipia felis	-	-
Salmonella infantis (8)	+	-	Vibrio cholerae	-	-
Salmonella newport (2)	+	-	Eikenella corrodens	-	-
Salmonella alachua	+	-	Pasteurella multocida	-	-
Salmonella javiana	+	-	Campylobacter jejuni	-	-
Salmonella havana	+	-	Serratia (3)	-	-
Salmonella senftenberg	+	-	Mesorhizobium haukuii	-	-
Salmonella anatum	+	-	Rhizobium sp	-	-
Salmonella saint paul	+	-	Bartonella henselae	-	-
Salmonella berta	+	-	Bartonella quintana	-	-
Salmonella braenderup	+	-	Corynebacteria (3)	-	-
Salmonella java (2)	+	-			

Total Organisms tested

274

This technology can be used to confirm the identity of isolates suspected to represent *Salmonella*, which would replace biochemical testing. This assay could rapidly identify the stool specimens that contain *Salmonella* (approximately 2% of stools submitted for culture), but more importantly would rapidly identify stool specimens that did not contain *Salmonella*, which would save labor and materials. This assay could also be used by the food and veterinarian industries for the rapid identification of *Salmonella* in food products and animals, respectively(3). The identification of *S. typhi* by melt point analysis is an attractive feature, since *S. typhi* causes enteric fever (a systemic illness with high mortality), as opposed to enteritis (a diarrheal disease), the most common form of salmonellosis in the United States. The *S. typhi* PCR could be used to confirm a *S. typhi* melting point obtained from the pan-*Salmonella* assay, to screen travelers or immigrants for colonization by *S. typhi*, or by the food or water industry, especially in developing countries where *S. typhi* is endemic.

SEQ. NO. 1Prgk gene 4139-4897 (underlined) Genbank No. AF008831

4081 atggatatac gataacggat caaaaatgat tctttgccag ataatgggta atggctgcct  
 4141 attcatttga cgatttcgcc ttatcatcag ccgttatgcc tttcttattg cgggcataat  
 4201 ggtttttcta ataccagacg ccaaagcctg ctgacatcac ggataacaaa ataataaaaa  
 4261 caatccaact ggttgcaaaa gaattacgtt ttactggtgt gccgggagcc tgtaattggg  
 4321 catcagaacg ttctgacaac acaacagaaa tgttgtcata atccacatcg gcaaaactat  
 4381 tctttaagaa acgcttgata tcgctgatct gatgcgcaag cggcgaacct cgttcatata  
 4441 cggctaattg cgacagatga acaggttttg gcggggcgcc attttcacca gcatcaatat  
 4501 cataactaat atggaccctg gcggagagca cgccctccat cgtctgtaat gactgttcca  
 4561 gtcgtgttc aatagccgaa tataacctgg ccttttcagc tcgaggagac gataccagcg  
 4621 aatccgccgg gaacatctgc gctatttcca ccgctggccg gggaggaagc tgataagttt  
 4681 taatccagta caccgcagcg gtaaaatcag gctcagcaac ggtaatgcta tagcccaatt  
 4741 ttccgctatc aattttattc gcctctatat tgtgcatttg cagaacggca atgacctcat  
 4801 tagcctgttc ctggtccagt ccttttaaaa gatccttacc cttacagccg gcaagggtca  
 4861 ttaccagcag aaaagtatat agatatcgac gaatcatgag cgtaatagcg tttcaacagc

SEQ. NO. 2VexC gene 41900-4295 (underlined) Genbank No. AL627283

41881 ctatccgtat atttactaat talatatcaa aggaataatc ttcagtttga atcgctgcct  
 41941 gatttgactg atattgttca aaaagcgcaq ttgcctgcgc taaatcttca cacattgtaa  
 42001 tttttccatg cagcagtacg ccaaagcgaat gacagtgttc ttttaataagc cgagggttgt  
 42061 gcgttagcac aatcaacctt ttctgttgca gctgacaagc tagcgccgcc tgcatacgca  
 42121 actgtgtggc attatccccg gtgtacagct tgccatcagc aatataaagg cggcagggaa  
 42181 gcagtaaat aatggcaaat gccagatgcg ttttcatcgt gacagaatat tcaactaccc  
 42241 tgctggtata gcaactgttca agctgggtca attgataaca gaaatglgaa aactcatcgc  
 42301 catccaggcc atatagactt gccatcattc gcgcattctc ctcaccggtt aaccctggca  
 42361 gaataaaaaga gtttgccccc agtgggagcg catcaccccg tagcccaata aaatccccct  
 42421 catcaggagc atccaggcca cacagcaacc tggttagcgt cgttttgcct gaaccaggag  
 42481 ccgccagaat accgatatgc tcatggtagc ccatgacaaa atctgtttta tcaaggacca  
 42541 cccgcgggcc cttatcagac tcaaaatagc gtgtacaacc taataaaccg aacacgaatg

Having described the invention, I claim the following:

1. A portion of nucleic acid that is useful for detection of all *Salmonella* strains and differentiation of these strains from other bacteria.
2. A portion of nucleic acid containing genetic information useful for the differentiation of *S. typhi* from non-Typhi *Salmonella*.
3. A real-time PCR assay that detects a specific portion of the organism's genome, a portion of the *prgK* gene, which is useful for the detection of all *Salmonella*, differentiation of *Salmonella* from other bacteria, and differentiation of *Salmonella* into Typhi and non-Typhi groups by melt curve analysis.
4. A portion of nucleic acid that affords the specific detection of only *S. typhi* from all other bacteria, including other *Salmonella* isolates.
5. A real-time PCR assay that detects only *S. typhi*, by the detection of the *vexC* gene, a portion of the gene complex associated with the Vi antigen.

60466398.D42903

File: C:\LightCycler3.2\Users\Administrator\Data\Salmonella-9-30-02\pan-salm-vs-bacti-batt-FAST Program: Amplification Run By: Administrator  
Run Date: Sep 30, 2002 14:29 Print Date: October 15, 2002

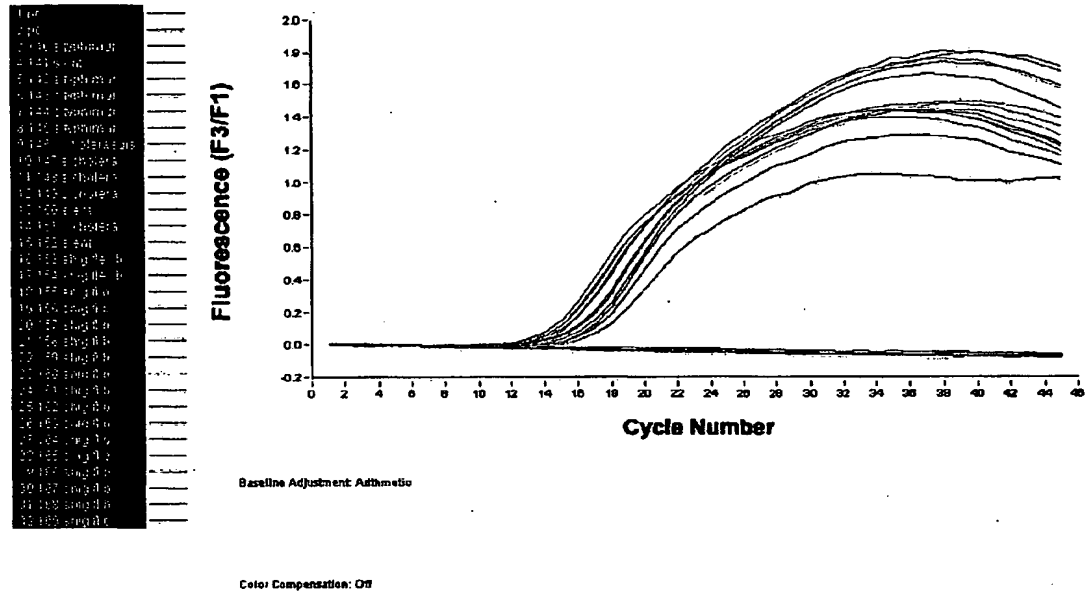


FIG. 1



SD466398.DV2903

File: C:\LightCycler3.2\Users\Administrator\Data\Salmonella9-30-02\pan sal m vs becd batt 5.ABT Program: Melt Run By: Administrator  
Run Date: Sep 30, 2002 12:43 Print Date: October 15, 2002

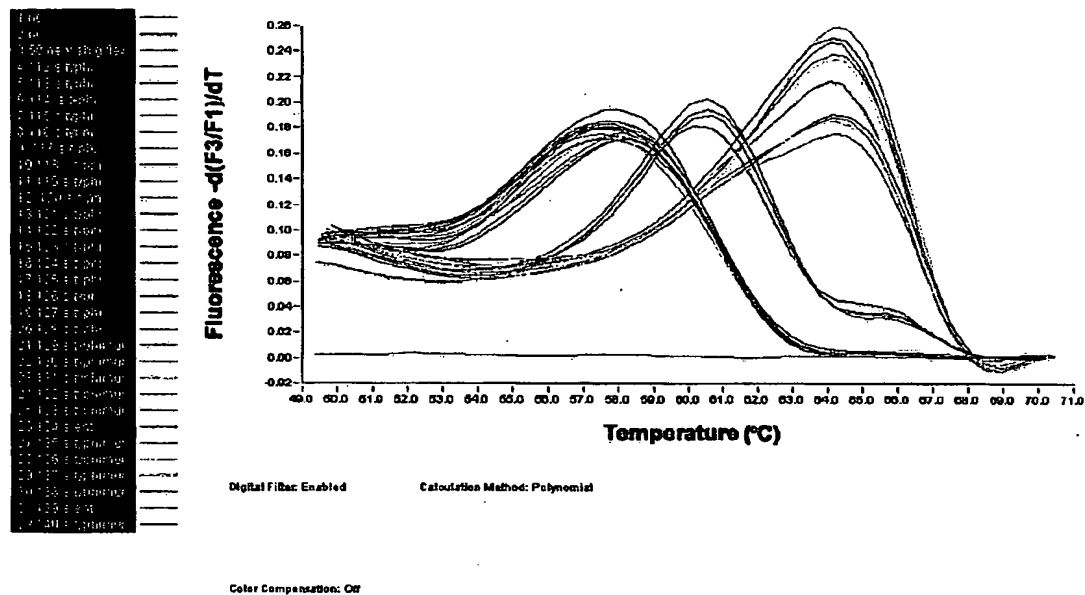


FIG. 2



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Organisation Mondiale de la Propriété Intellectuelle (OMPI) - Genève, Suisse

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I hereby declare that I believe I am the original, first and sole (if only one inventor is listed below) or joint (if more than one inventor is listed below) inventor of the subject matter which is claimed and for which a patent is sought.

This declaration is directed to the international application of which it forms a part (if filing declaration with application).

This declaration is directed to international application No. PCT/..... (if furnishing declaration pursuant to Rule 26ter).

I hereby declare that my residence, mailing address, and citizenship are as stated next to my name.

I hereby state that I have reviewed and understand the contents of the above-identified international application, including the claims of said application. I have identified in the request of said application, in compliance with PCT Rule 4.10, any claim to foreign priority, and I have identified below, under the heading "Prior Applications," by application number, country or Member of the World Trade Organization, day, month and year of filing, any application for a patent or inventor's certificate filed in a country other than the United States of America, including any PCT international application designating at least one country other than the United States of America, having a filing date before that of the application on which foreign priority is claimed.

Prior Applications: 60/466,398, US, 29 April 2003

I hereby acknowledge the duty to disclose information that is known by me to be material to patentability as defined by 37 C.F.R. § 1.56, including for continuation-in-part applications, material information which became available between the filing date of the prior application and the PCT international filing date of the continuation-in-part application.


I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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 (if not contained in the request, or if declaration is corrected or added under Rule 26ter after the filing of the international application. The signature must be that of the inventor, not that of the agent)

Date: 4/22/03  
 (of signature which is not contained in the request, or of the declaration that is corrected or added under Rule 26ter after the filing of the international application)

Name: .....

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Inventor's Signature: .....  
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